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DNA Damage Following Acute Aerobic Exercise: A Systematic Review and Meta-Analysis

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Abstract

Background: Exercise is widely recognised for its health enhancing benefits. Despite this, an overproduction of reactive oxygen and nitrogen species (RONS), outstripping antioxidant defence mechanisms, can lead to a state of (chronic) oxidative stress. DNA is a vulnerable target of RONS attack and, if left unrepaired, DNA damage may cause genetic instability.

Objective: This meta-analysis aimed to systematically investigate and assess the overall effect of studies reporting DNA damage following acute aerobic exercise.

Methods: Web of Science, PubMed, MEDLINE, EMBASE and Scopus were searched until April 2019. Outcomes included (1) multiple time-points (TPs) of measuring DNA damage post-exercise, (2) two different quantification methods (comet assay and 8-Oxo-2'-deoxyguanosine; 8-OHdG), and (3) protocols of high-intensity ($\geq 75\%$ of maximum rate of oxygen consumption; VO_{2-max}) and long-distance (≥ 42 km).

Results: Literature search identified 4316 non-duplicate records of which 35 studies were included in the meta-analysis. The evidence was strong, showcasing an increase in DNA damage immediately following acute aerobic exercise with a large effect size at TP 0 (0h) (SMD=0.875; 95% CI:0.5,1.25; $p < 0.05$). When comparing between comet assay and 8-OHdG at TP 0, significance was observed only when using the comet assay. Finally, when isolating protocols of long-distance and high-intensity exercise, increased DNA damage was only observed in the latter. (SMD=0.48; 95% CI:-0.16,1.03; $p = 0.15$ & SMD=1.18; 95% CI:0.71,1.65; $p < 0.05$ respectively).

Conclusions: A substantial increase in DNA damage occurs immediately following acute aerobic exercise. This increase remains significant between 2 hours - 1 day but not 5-28 days post-exercise. Such an increase was also not observed in protocols of long-distance. The relationship between exercise and DNA damage may be explained through the hormesis theory, which is somewhat one-dimensional, and thus limited. The hormesis theory describes how exercise modulates any advantageous or harmful effects mediated through RONS, by increasing DNA oxidation between the two end-points of the curve: physical inactivity and overtraining. We propose a more intricate approach to explain this relationship: a multi-dimensional model, to develop a better understanding of the complexity of the relationship between DNA integrity and exercise.

Key Points

- Acute exercise can damage single-stranded DNA, and DNA repair likely occurs within at least 3 days.
- Multiple factors affect the extent of exercise-induced DNA damage and its repair.
- An elaborate, multi-dimensional approach should be considered to fully understand the complex relationship between exercise, Reactive Oxygen and Nitrogen Species and DNA damage.

1 Introduction

Exercise is widely regarded as a primary conduit to a proficient state of health, and there is now ample evidence from both observational studies and randomised trials to postulate that regular exercise is a contributing factor in the prevention of cardiovascular disease, cancer, diabetes and other chronic conditions, as well as reducing the risk of all-cause mortality [1,2].

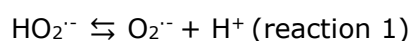
Despite this paradigm, multiple studies have established a link between strenuous and/or exhaustive exercise, and the increased formation of reactive oxygen and nitrogen species (RONS) [3]. RONS are generated endogenously in most aerobic organisms by an incomplete reduction of oxygen, and mainly via the mitochondrial electron transport chain during cellular respiration [4]. It is currently well-understood that between 0.12-2% of the oxygen utilised by mitochondria during normal respiration is not converted to water (tetravalent reduction), but instead is reduced to the superoxide anion ($O_2^{\cdot-}$), which can subsequently be reduced to hydrogen peroxide (H_2O_2) and further to the more potent hydroxyl free radical (OH^*) [5,6]. However, of note, the percentage estimation of total oxygen consumption in mitochondrial RONS production refers primarily to the *in vitro* based experiments performed by Chance and colleagues [7]; as such, the production of $O_2^{\cdot-}$ *in vivo*, may indeed be much less [8–10].

RONS are often implicated in complex molecular mechanisms designed to explain the process of human ageing and associated chronic diseases states [11]. Associated molecules such as lipid, protein and DNA are known vulnerable targets of RONS attack, and therefore can be oxidatively modified [12]. Oxidative free radical attack and subsequent damage to DNA, in particular, is of prime biomedical importance and interest, as if left unrepaired, significant DNA alterations (e.g. chromosomal rearrangement, base damage, and strand breaks) may lead to rapid ageing, mutagenesis, and ultimately carcinogenesis [13–15]. Paradoxically, although excessive RONS production may be implicated in the pathology of

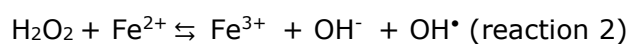
numerous diseases [16], when produced in moderate/low amounts (i.e. not inducing a state of oxidative stress defined as an 'imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage') [17], they act as key intracellular signalling molecules regulating a host of physiological and biological processes [11,18]. RONS are generated in skeletal muscle and play a key role in skeletal muscle adaptation to aerobic exercise training [19,20]. *In vitro* work has shown myotubes exposed to hydrogen peroxide (exogenous), increases the expression of peroxisome-proliferator-activated receptor (PPAR- γ), and peroxisome-gamma co-activator-1 alpha (PGC-1 α), whereas further exposure to N-acetylcysteine, an antioxidant, impeded its activity[21]. PGC-1 α , which is induced by AMP kinase (AMPK), is a signalling pathway involved in adaptation to endurance exercise leading to mitochondrial biogenesis [18]. Similarly, *in vivo* work has demonstrated that antioxidant supplementation can hinder essential training adaptation mechanisms in humans. A study administering 1 g of vitamin C per day to humans during 8 weeks of training (3d/week at 65%to 80% VO_{2-max} ; 5% increase every 2 weeks), resulted in decreased expression of PGC-1 α and mitochondrial transcription factor A, both of which are key transcription factors involved in mitochondrial biogenesis [22].

Mechanistically, there are several ways free radicals can be generated during exercise. While exercising, the energy requirements in the body greatly increase, leading to a substantially higher rate of oxygen uptake up to 15-fold, and in active muscle, the oxygen flux may increase to about 100-fold compared to resting values [23,24]. The primary radical species produced by the contracting skeletal muscle are $O_2^{\cdot-}$ and nitric oxide (NO) [25]. When electron transfer occurs normally through the electron mitochondrial transport chain to reduce oxygen to water, about 1-3% of all electrons are leaked resulting in the formation of $O_2^{\cdot-}$ by adding one electron to molecular oxygen [13,26]. Apart from the mitochondria, there are enzymatic sources that contribute substantially to free radical production such as nicotinamide

adenine dinucleotide phosphate (NADPH) oxidase, the enzyme which catalyses the one electron reduction of molecular oxygen (reaction 1) upon the activation of phagocytosis [13,27].



Central to these mechanisms is the generation of the superoxide anion (O_2^- ; one-electron reduction) and subsequently, produced through the superoxide dismutases (SODs), hydrogen peroxide (H_2O_2 ; two-electron reduction) [13]. Following the production of H_2O_2 , the hydroxyl radical (OH^\bullet ; three-electron reduction) can be produced in the presence of transition metal catalysts, through the Haber-Weiss Fenton reaction (reaction 2) [13,26]. O_2^- also reacts with NO (reaction 3) to produce peroxynitrite (ONOO^-), a highly reactive RONS that can cause damage to DNA and nitrate proteins [26].



As RONS accumulate in the cell, either from metabolic signalling (NADPH) pathways or external sources, they are balanced by scavenging antioxidant systems [28]. Under these balanced conditions, RONS are used as signalling molecules or, under unbalanced conditions, can interact with Fe^{2+} through Fenton chemistry, as mentioned above, and cause cellular damage due to hydroxyl radicals (OH^\bullet), which in turn can be attenuated by DNA repair mechanisms. In the case of over-accumulation of such DNA damage and insufficient repair, it is conceivable to suggest that rapidly dividing cells may promote a mutational profile leading to disease. However, per their signalling role, RONS and DNA damage can trigger physiological programmed cell death (apoptosis) by activating p53 to prevent mutagenesis/carcinogenesis [28]. Therefore, it is important to differentiate whether cell death is caused by oxidative stress (i.e. DNA damage), which can be avoided (scavenging systems, DNA repair mechanisms), or programmed cell death via RONS signalling which could be advantageous when the cell becomes compromised, as a result of DNA damage[28]. RONS are therefore important

molecules involved in the fate of the cell's destiny as they regulate crucial processes such as growth, differentiation, and cell death [29]. Once DNA is damaged, it is normally repaired by mechanisms such as base excision repair (BER), nucleotide excision repair (NER), or through a process of homologous recombination (HR) or nonhomologous end joining (NHEJ); the type of DNA repair will depend on the mechanism and the extent of the damage [30] (**Electronic Supplementary Material Figure S1**).

Exercise represents an intriguing model to examine the dynamic role of RONS from both a physiological and pathological perspective. Evidence suggests that only exhaustive (long-distance) and/or strenuous exercise (high-intensity maximal exercise, marathons, triathlons and overtraining) can induce detrimental DNA alterations, if left unrepaired [18,31]. However, during low or moderate intensity and distance exercise, the generated RONS may serve to act as signalling molecules responsible for the initiation of exercise and skeletal muscle adaptation [19,31,32], as often conceptualised through the hormesis theory.

The aim of this work is to systematically investigate data reporting DNA damage following acute aerobic exercise, and perform a meta-analysis to examine the overall effect from these studies. There are discrepancies regarding exercise intensity and that it necessarily needs to be very exhaustive/strenuous to cause oxidative damage and/or stress and this review will aim to elucidate this. Furthermore, the possible physiological and/or pathological consequences of exercise-induced DNA damage need to be considered in relation to the exercising individual in line with a new proposed multi-dimensional model. This is the first meta-analysis aimed to investigate the relationship between DNA damage and exercise.

2 Methods

2.1 Search Strategy

According to the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines [33], a detailed search was conducted to identify all relevant studies (including a range of publication from 1900 – April 2019) across the following five databases: Web of Science, PubMed, MEDLINE, EMBASE, and Scopus. Searching was limited to articles published in English and the filter “in humans” was applied on PubMed, MEDLINE and EMBASE.

2.2 Inclusion/Exclusion Criteria

All published studies were checked for the following criteria: (1) the study was a full report published in a peer-reviewed journal; (2) the study assessed humans; and (3) the keyword combination referred to the following terms (used in all possible combinations): *exercise, exercis*, exercise training, endurance, exhaustive, exercise-induced, acute exercis*, physical activity, DNA, nucleoid DNA, deoxyribonucleic acid, 8-hydroxy-2'-deoxyguanosine, 8-hydroxy-2-deoxyguanosine, 8-oxo-7,8-dihydro-2'-deoxyguanosine, 8-oxo-7,8-dihydro-2-deoxyguanosine, 8-hydroxy-2-deoxy guanosine, 8-hydroxydeoxyguanosine, 8-oxoguanine, 8-hydroxyguanosine, 8-oxo-2-deoxyguanosine, 8-OHdG, 8OHdG, 8-OH-dG, 8-OHG, 8-oxo-dG, 8-oxodG, 8-Oxo-dG, 8-oxo-G, damage, oxidative damage, oxidative stress*. Note that for the purposes of this review we used the term *DNA damage* to encompass DNA single strand breakage and nucleotide base oxidation.

One investigator initially reviewed records generated from all databases and applied the inclusion/exclusion criteria to identify eligible studies for inclusion; these were then agreed with at least three of the authors. The inclusion/exclusion criteria are shown in **Table 1**. To note, acute exercise was defined as aerobic

exercise performed over a short period of time but could also extend up to 1-3 days of a marathon event. To minimise the limitation of various biological samples, studies utilising urine, red blood and muscle cells were also excluded. Please see **Electronic Supplementary Material Table S1** for information and detail of excluded studies.

2.3 Data Extraction

A general extraction form was used, once the number of included studies was finalised. Characteristics of the participants (sample size, age, and sex), the exercise protocol (distance and intensity), assayed biomarkers, and methods of DNA quantification used were extracted by one investigator. The outcome measure, DNA damage, was expressed using multiple descriptors, and with regard to the comet assay these were: DNA in the tail (%); DNA migration (μm) (otherwise known as tail length); tail moment (also known as olive tail moment) which is the product of tail (%) and tail length [34]. The biomarker used was 8-OHdG. Due to variations in the analytical approach, high performance liquid chromatography (HPLC) or enzyme-linked immunosorbent assay (ELISA), 8-OHdG (pg/ml) and 8-OHdG/ 10^5 dG are also reported. The tail DNA (%), DNA migration (μm) or tail length and tail moment correspond to the comet assay and the 8-OHdG (ng/ml) or (pg/ml) and 8-OHdG/ 10^5 dG to HPLC or ELISA methods. In reference to the comet assay, where multiple image descriptors were reported by one study, the authors used tail (%), as this is regarded as the most sensitive descriptor/parameter compared to tail moment or length [35]. Data were collected as means and standard deviation (SD) or standard error of the mean (SEM). Graph digitizer software (DigitizeIt, Braunschweig, Germany) and WebPlotDigitizer (Web Plot Digitizer, V.4.2. Texas, USA: Ankit Rohatgi, 2019) were used to obtain data from studies where data were only presented in a figure format. In two studies [36,37], data were not extractable and therefore not included in the meta-analysis.

Numerous studies included heterogeneous groups of participants: trained or untrained, young or old, sport-specific volunteers (such as swimmers, rowers, runners), physically active and sedentary participants and a few studies compared men and women. Furthermore, three studies [38–40] used more than one parameter to quantify DNA damage. Lastly, some studies measured DNA damage at only one time-point (TP) while other studies included multiple post exercise measures of DNA damage following exercise. **Table 2** details corresponding TPs for each investigation.

2.4 Data Analysis

The primary outcome was defined as DNA oxidative damage before and following exercise at TP 0 (0h) grouped by method of DNA damage quantification (1) comet assay and (2) 8-OHdG. Secondary outcomes included: (3) high-intensity ($\geq 75\%$ of maximum rate of oxygen consumption; VO_{2-max}) and (4) long-distance ($\geq 42\text{km}$) as different exercise protocols all measured, and finally (5), DNA damage at further time-points 1-11 (ranging from 15 min-28 days).

2.5 Quality Assessment

In order to assess the quality of included studies, the risk of bias was assessed by one investigator using the 12 criteria (rating: yes, no, unsure) recommended by the Cochrane Back Review Group (**Table 3**) [41]. The criteria assess risk of bias using the five following categories: selection bias; performance bias; attrition bias; reporting bias and detection bias. However, due to the inherent difficulties in blinding participants to exercise treatments, seven of the twelve criteria were not applicable, and as such not included. These were: adequate method of randomization; allocation concealment; outcome assessor blinding; participant and provider blinding; similarity or not of co-interventions and intention-to-treat analysis. In contrast, two additional sources of bias, smoking and training status, were included as criteria given their potential to influence exercise responses.

Following these modifications, the maximum score that studies could gather would be seven, with the lowest scores indicating high risk of bias and higher scores indicating lower risk of bias. To establish a clearer overall assessment of bias, a high, moderate and low risk scale was developed according to how studies scored. Therefore, the following ranges were developed: 1-3 = high risk, 4-5 = moderate and 6-7 = low risk.

2.6 Statistical Analysis

Assessment of effect size: Meta-analyses were calculated using Comprehensive Meta-Analysis (Version 3.3.070, NJ:USA: Biostat, Inc). A random effects model was used since it assumes statistical heterogeneity among studies and that studies represent a random sample of effect sizes that could have been observed [42,43]. Standardised mean differences (SMD) adjusted with Hedges' g and 95% confidence intervals (CI) were calculated as the difference in means before and after exercise divided by the pooled standard deviation [43]. Where studies did not report standard deviations, these were calculated from standard errors [42]. The SMD measure was used to express effect size, the magnitude of which was calculated using Cohen's categories: (1) small: SMD=0.2-0.5, (2) medium: SMD=0.5-0.8 and (3) large: SMD>0.8 [44,45]. A positive SMD measure was considered to show increased DNA damage after exercise compared to rest, whereas a negative SMD measure would show greater DNA damage at rest in comparison to after exercise. The overall effect was assessed using Z scores with a set significance level of $p < 0.05$.

Assessment of heterogeneity: The χ^2 Cochran's Q test and the I^2 statistic were used for the assessment of statistical heterogeneity among studies. The χ^2 test assesses whether the observed differences in results are compatible with chance alone and a p value ≤ 0.10 was considered to display significant heterogeneity [42]. Furthermore, the I^2 statistic was used to quantify inconsistency across studies, with (1) $I^2=0-30\%$ showing no heterogeneity, (2) $I^2=30-49\%$

showing moderate heterogeneity, (3) $I^2=50-74\%$ showing substantial heterogeneity and (4) $I^2=5-100\%$ showing considerable heterogeneity [42].

Subgroup and Sensitivity Analysis: Subgroup analyses were performed for multiple time-points of DNA damage quantification after exercise grouped by different methodologies in DNA quantification (comet assay versus 8-OHdG), and according to the exercise protocol: high-intensity ($\geq 75\%$ VO_{2-max}) versus long-distance (≥ 42 km). To assess the robustness of the significant outcome data, sensitivity analysis was planned by excluding studies with high risk of bias.

Publication Bias: Publication bias was assessed, when at least 10 studies were included in the meta-analyses, by visually analysing funnel plots. Generally, asymmetrical funnel plots were considered to indicate high risk of publication bias, while symmetrical funnels plots were considered to indicate low risk [46].

3 Results

3.1 Literature Search

The number of articles identified from all electronic database searches and the selection process is shown in **Figure 1**. Four thousand four hundred and twenty records (4,420) were retrieved in the database search, one hundred and four (104) of which were duplicates. Four thousand one hundred and forty-one articles (4,141) were excluded after title screening, leaving one hundred and seventy-five (175) records for abstract screening. One hundred and thirteen (113) records were excluded after abstract screening and sixty-two (62) full-text articles were assessed for eligibility. Twenty-three (23) full-text articles were excluded due to various reasons (detailed in **Electronic Supplementary Material Table S1**). The most common reason for study exclusion was the exercise protocol not consisting of acute and aerobic exercise. Thirty-nine studies (39) were included in the qualitative analysis, out of which one (1) was excluded due to same sample size

[47], one had unpublished data [48] and two (2) due to non-extractable data [36,37] and therefore thirty-five (35) were included in the quantitative analysis.

3.2 Study Characteristics

The characteristics of each study including participants, exercise protocol, sample source, biomarker, quantification technique and results are presented in **Table 4**.

Participants: Participant age ranged from 18 to 70 years old. Five studies included both male and female participants [49–53]. Three studies included groups of untrained and trained subjects [36,39,54], one study [55] used rowers and physical education students, while another [56] used swimmers and runners. Finally, two studies used volunteers participating in multiple running distances [40,49].

Biomarkers/Analytical Techniques: With regard to the biomarker and the techniques used to quantify DNA damage, twelve studies used 8-OHdG [36,50,53,56–64] with either HPLC or ELISA. A total of twenty-seven studies used tail DNA (%) or strand breaks, tail length, tail moment with the comet assay technique [37–40,48,49,51,52,54,55,65–80].

Exercise Protocol: There was variation in the chosen exercise protocols, most often involving treadmill exercise and cycling whilst employing different exercise intensities (ranged from 40%-100% VO_{2-max}). Eight studies included marathons, half-marathons or ultramarathons [40,49,51,69,71,75,76,79] and three studies [47,77,79] involved a triathlon as part of the exercise protocol.

Quality Assessment in Individual Studies: No study scored in the high-risk bias range (1-3), eleven studies scored in the moderate risk range (4-5), and the remaining twenty-seven studies scored in the low risk range (6-7).

3.3 Analysis of Overall Effects

In summary, as seen in **Table 5**, a significant increase in DNA damage following exercise was observed at time-points 0 (0h), 2 (2h), 3 (3h), 4 (4-6h), 5(1d) and 7 (3d). No significances were found at time-points 1 (15min-1h), 6 (2d), 8 (4d), 9 (5d), 10 (6-7d) and 11 (14-28d).

Overall effect of DNA damage after exercise at TP 0

For DNA damage after exercise at TP 0 (0h), data were available from 24 studies, with a total number of 312 participants. As seen in **Figure 2**, compared to rest, there was a significant increase in DNA damage after exercise (SMD=0.875; 95% CI:0.5,1.25; $p<0.05$). Heterogeneity among studies was found to be considerable ($\chi^2=5.25$, $p=0.02$, $I^2=82.12\%$).

Comet assay vs 8-OHdG at TP 0

Similarly, as shown in **Figure 2**, for studies utilizing only the comet assay with 203 participants, DNA damage remained significantly higher after exercise at TP 0 (0h) compared to rest (SMD=1.14; 95% CI:0.7,1.58; $p<0.05$). Moreover, although the number of studies using the 8-OHdG biomarker was considerably less, with 109 participants, no change in DNA damage compared to rest using this assay was observed. (Please see **Figure 2**; SMD=0.15; 95% CI:-0.58, 0.88; $p=0.68$).

High-intensity Exercise ($\geq 75\%$ VO_{2-max})

DNA damage was increased after high-intensity exercise ($\geq 75\%$ VO_{2-max}), measured at time-point 0 (0h) & 5 (1d) (**Figure 3a**; SMD=1.18; 95% CI:0.71,1.65; $p<0.05$; heterogeneity: $\chi^2=3.1$, $p=0.08$, $I^2=63.98\%$).

Long-Distance Exercise (≥ 42 km)

As shown in **Figure 3b**, DNA damage was not significantly higher after long-distance (≥ 42 km) exercise at time-point 0 (0h) & 1 (15min-1h) (SMD=0.48; 95% CI:-0.16,1.03; $p=0.15$; heterogeneity: $\chi^2=25.84$, $p=0.001$, $I^2 = 72.91\%$).

3.4 Summary of Findings

Using data from 35 studies and 312 participants, this paper quantitatively demonstrates, for the first time, that DNA damage increases immediately following acute aerobic exercise (**Figure 2**). Based on Cohen's classification, the effect on DNA damage was large (>0.8). No significances were seen 1 hour (**Figure 4a**), however, increased DNA damage was observed from 2 hours to 1 day following exercise (**Figure 4b,4c, Electronic Supplementary Material Figure S2a and S2b**). Similarly, no DNA damage was observed 2 days following exercise (**Figure S3a**) but significantly increased 3 days post exercise (**Electronic Supplementary Material Figure S3b**). Furthermore, when comparing the two methods of DNA assessment (comet assay and 8-OHdG), significance was observed only in studies using comet assay, at time-point 0 hours, 3 hours and 1 day, again with a large effect size. No differences were observed 5-28 days post-exercise (**Electronic Supplementary Material Figure S4a, S4b and S4c**). Finally, when isolating protocols of high-intensity ($\geq 75\%$ VO_{2-max}) and long-distance (≥ 42 km), greater DNA damage following exercise was observed only in the former (**Figure 3a and 3b**). However, it should be noted that, no long-distance study in our analysis used 8-OHdG as a biomarker for oxidative damage. Whereas, in the high-intensity protocols, a mixture of both methods was utilized. As it has been suggested [51,77], DNA damage measured after long-distance exercise (7-10 hr race) may not be detected due to the activation of repair mechanisms and increased clearance of damaged cells initiated during the race, which would otherwise not be observed when measured after a shorter exercise protocol. Additionally, these processes could be further enhanced due to the intake of antioxidants ingested during the race.

4 Discussion

The main purpose of this meta-analysis was to examine the effect of acute exercise on DNA damage. These results suggest that exhaustive exercise leads to increased DNA damage. Acute aerobic exercise, regardless of intensity, seems to produce sufficient stimulus for a greater production of RONS which may evoke damaging effects to DNA. After longer distance events, such as triathlons and ultramarathons, added protection against DNA damage may be offered through the initiation of repair systems and adequate antioxidant intake from food/drinks consumed during such events; however more studies are needed to confirm this.

4.1 Mechanisms of Free Radical Production During Exercise

Previous work from our laboratory has shown increased DNA damage of 63% following exercise as well as a greater concentration of H₂O₂ as a function of exercise, compared to rest, indicating a possible mechanism of exercise-induced DNA damage through the increased production of H₂O₂ [66]. Moreover, the activation of inflammatory cells such as neutrophils and lymphocytes during exercise, due to muscle tissue damage, can further enhance superoxide production, which can cause direct damage to DNA [75,80]. Additionally, catecholamines released during exercise can be autoxidized and lead to the production of non-radicals such as H₂O₂ [81]. Finally, during high intensity aerobic exercise, tissue ischaemia occurs, resulting in an increased number of hydrogen ions which can in turn react with superoxide anions to produce further RONS [82].

4.2 Free Radical-Induced Damage to DNA/Repair

Although O₂⁻ and NO are the primary radical species produced by contracting skeletal muscle, these do not directly cause damage to DNA [14]. Instead, OH[•] reacts with the different components of DNA, such as DNA bases and the deoxyribose sugar, causing damage either by hydrogen addition or abstraction, producing multiple products, as well as single- and/or double-strand breaks,

tandem lesions and DNA protein cross-links [14,83]. Among the four DNA bases, guanine has the least reduction potential, and acts as an excellent electron donor and is the most prone to oxidation by OH^{*} [83]. For this reason, the product 8-OHdG is the most popular biomarker of DNA damage in urine and blood samples [12]. Furthermore, compared to guanine, adenine has a greater reduction potential and is not oxidized to the same extent [83]. Just as with guanine, OH^{*} reacts with adenine by adding a hydrogen molecule to its double bonds at specific locations but in a slightly different distribution to that of guanine [83]. The base excision repair pathway is normally activated to repair DNA damage, and this occurs following the activation of a number of enzymes such as DNA glycosylase-1 [84], endonuclease phosphodiesterase and DNA polymerase [85]. Repair to DNA is almost always controlled by a number of factors such as availability of said enzymes and others such as p53 and RAS [86,87].

4.3 Hormesis Theory

The relationship between exercise, RONS and DNA damage has been explained in the context of the hormesis theory (displayed in **Figure 5**) [18]. In toxicology, hormesis refers to an environmental agent's beneficial effect on a cell or organism at low doses that is otherwise harmful at high doses, creating a bell-shaped curve [88]. In this instance, exercise acts as the stimulus and the subsequent effects of exercise-induced RONS (physiological or pathological) are determined by the dose. Being physically inactive is a major risk factor for numerous chronic diseases and physiological disorders such as cancer, type 2 diabetes, cardiovascular disease, metabolic syndrome, hypertension and obesity [89–91]. In 2000, physical inactivity in combination with poor diet was the second leading cause of death after tobacco in the US, contributing to 16.6% of total US deaths [92].

Physical inactivity represents one end-point of the hormesis curve, while overtraining and strenuous unaccustomed/unindividualized exercise represents the

opposite end-point; both result in a higher risk of disease and decreased physiological function and are mediated by elevated RONS production and oxidative stress [93]. Regular exercise can lead to adaptation through up-regulation of molecular and cellular pathways, redox signalling and antioxidant repair systems, resulting in the enhanced capacity of the organism to overcome greater stress [11,93]. In addition, exercise training can further extend that adaptive response by 'stretching' the capacity to tolerate even higher levels of RONS [93]. Yet, when RONS production outstrips antioxidant defence mechanisms and there is insufficient repair of DNA double strand breaks, this can cause chromosome instability and gene mutation can occur [15,94]. However, it is unclear where the threshold limit exists between the beneficial effects of regular exercise and the point of overtraining associated with higher oxidative DNA damage and insufficient repair. This makes the concept of hormesis definitive but narrow. Defining this point is inherently complex due to the heterogeneous variations across individuals based on sex, age, fitness and exercise intensity and distance. However, along with these, there are even more complex factors (discussed in section 4.5) that influence the degree of the damage and therefore the overall effect of the beneficial adaptations and the harmful effects of the two end-points that should be considered.

4.4 One-dimensional vs Multidimensional Model

A role for RONS in exercise-mediated adaptations and responses is evident [95]. The concept of hormesis can allow us, to some extent, to understand how the relationship between exercise and DNA oxidation can fit into a bell-shaped curve. However, it only considers levels of RONS/DNA oxidation, rendering it somewhat one-dimensional. While this may be an important factor in explaining the fundamental adaptive responses to exercise, when investigating the extent of DNA oxidative damage, there are multiple factors to consider. We propose three more basic factors instead of only levels of RONS/DNA oxidation in a more intricate and adaptable multi-dimensional model, visualised in a radar chart starting from the

centre (least damaging) to the edge of the circle (most damaging) in a linear scale manner shown in **Figure 6**. Thus, this proposed multi-dimensional model would consist of the following four factors: (1) type of RONS, ranging from the least reactive (such as $O_2^{\cdot-}$) to the most reactive radical (such as OH^*); (2) frequency of RONS attacks/episodes, ranging from one to multiple episodes; (3) type/extent of DNA damage, either single- or double-strand breaks, ranging from the least to the most damaging effect and (4) magnitude of RONS/DNA oxidation, ranging from lowest to maximum levels of DNA oxidation/RONS increase. When applying this multi-dimensional model to the exercise stimulus, there are four more specific factors to consider: (5) exercise intensity/ distance; (6) exercise frequency; (7) sufficiency of DNA repair enzymes and (8) degree of individualization (sex, age, training level, nutrition quantification method) (**Figure 6**).

As exercise occurs, adaptive mechanisms are stimulated and these lead to the accentuation of antioxidant enzymes, as result of training adaptation [96]. However, if multiple individual sporadic bouts of acute, but not regular, exercise occur (effect of overtraining and/or excessive exercise resulting from very high-intensity and/or long-distance exercise), without sufficient rest periods in between, the repair systems most likely fail due to higher oxidative stress resulting from enhanced RONS production [96]. Successful adaptations are thus unlikely and detrimental health outcomes may occur as a consequence. In contrast, individual bouts of exercise with complete recovery in between could revoke any oxidative stress via the antioxidant enzymes which are upregulated within the muscle as a function of training, suggesting exercise itself can exert an antioxidant effect [97,98]. In turn, this supports the now established theory that RONS production is in fact a necessary step to stimulate the adaption of the skeletal muscle in response to exercise [19]. Furthermore, the severity of the damage and whether genome stability is being compromised or not, depends on the type of damage/oxidation that has occurred to the DNA – base oxidation, single or double strand breaks.

Cumulatively, these factors can affect the degree of DNA oxidative damage by causing the least to the most amount of DNA damage and, in turn, possibly creating (individual) different individual thresholds for the end-points of physical inactivity and overtraining and the way the hormesis effect unfolds as a bell-shaped curve. Obviously, a combination of reaching the higher end of the scale in all factors (towards the circumference of the circle) would result in the most harmful kind of oxidative DNA alteration, compared to the lower end of the scale (towards the centre of circle) where the outcome may be less harmful.

This meta-analysis suggests that aerobic exercise leads to an increase in oxidative DNA damage as measured by the comet assay. It is important to elucidate what this means in relation to health outcomes. The literature collectively suggests that a single acute bout of exercise (even of high-intensity/long-distance) is not likely able to cause any long-term and significantly harmful effects as explained under the hormesis theory. Ironman triathlon studies have shown that well-trained athletes show a large decline in DNA damage post-race. For instance, Mastaloudis *et al.* reported that DNA damage decreased below baseline levels 2 days after a 50-km ultramarathon [51]. Moreover, an 8% decrease below baseline was observed after 6 days. Similarly, Wagner *et al.* showed that levels of DNA damage after an Ironman triathlon returned to baseline values 5 days after the event, suggesting non-persistent DNA damage [77]. This can be attributed to the up-regulation of repair mechanisms and enhanced endogenous antioxidative system which indicates that endurance training can enhance the body's ability to prevent and repair DNA damage, largely by increasing its antioxidant defenses [77]. The non-trained cohorts may not have that added antioxidant protection as a function of adaptive training. Master endurance athletes are also shown to have longer telomere length (TL), a marker of biological age, than non-athlete age-matched controls [99,100]. Telomeres are responsible for stopping cell division by activating DNA damage recognition systems [101]. TL shortening has been shown to be attenuated by long-term endurance training and thus, reduced antioxidant activity

and accumulation of RONS may contribute to TL debilitation [100]. Taking this into account, along with the use of different experimental designs, fitness levels and methods of DNA damage detection at various post exercise TPs, all these factors may affect the degree of the damage and the extent to which it is (efficiently) repaired.

In summary, both the advantageous and harmful effects of exercise-associated adaptations and the two end-points, physical inactivity and overtraining, are results of non-exposure or repeated exposure to the stimulus (inactivity or repeated exercise bouts) combined with a varying degree of DNA oxidative damage. Whether or not physiological or pathological consequences occur, and to what extent, may depend on all factors mentioned in the multidimensional model.

4.5 Strengths and Limitations

This is the first meta-analysis available on DNA damage and exercise. DNA damage was distinguished while performing sensitivity analysis of two of the most common methods of quantification found across studies, the comet assay and 8-OHdG. The overall risk bias was low since studies scored well in the quality assessment table. Finally, PRISMA guidelines [33] and Cochrane collaboration recommendations were followed [42].

Some limitations have been identified in the included studies. A number of study data had to be manually extracted from figures due to data not being presented in the text. However, the degree of error should be minimal due to the high accuracy of the software used. Moreover, the sample size for the two quantification methods was not equal, and while this is expected given the variety of study methodologies used is nonetheless noteworthy. This may be the main reason why only studies utilising the comet assay showed significantly greater DNA damage following exercise as opposed to 8-OHdG. However, this could also result due to interlaboratory differences. In 2005, the European Standards Committee on Oxidative DNA Damage found no association between levels of oxidative DNA

damage in a sample of 88 healthy males measured by the comet assay and 8-OHdG by HPLC methods in six different laboratories [102]. Therefore, the validity and comparability of different methods of oxidative DNA damage across laboratories may be questioned. Similarly, the number of studies/sample size at all time-points and in the subgroup analysis (high-intensity and long-distance studies) varied and could explain the difference between observed significance and non-significance between the two protocols.

The authors chose to focus solely on studies that have quantified DNA damage assayed from blood as these represent the most frequently measured in the literature. Nevertheless, we acknowledge that DNA damage can also be determined in urine and muscle. Studies measuring DNA damage following exercise in tissues/specimens other than white blood cells (e.g., muscle and urine) support our data demonstrating that exercise induces DNA damage. Previous work from our laboratory shows an 86% increase, compared to rest, in muscle 8-OHdG concentration following 100 isolated and continuous maximal knee contractions [103]. Moreover, during a four-day race, urinary 8-OHdG of five super-marathon runners were monitored where after day one (93 km) 8-OHdG increased, on day two (120 km) no further increase occurred, while on days three and four (56 and 59 km respectively) there was a decrease in 8-OHdG suggesting the likelihood of exercise adaptation and upregulation of antioxidant systems [104]. Similarly, after 8 days of running (30 ± 3 km/day) at a training camp, 8-OHdG measured from urine increased significantly by 26% [105]. Another investigation showed that, after one hour of cycling at 70% of maximal O₂ uptake, urinary 8-OHdG was elevated, and this increase remained significant 1d post-exercise [106].

Furthermore, training status was not distinguished across studies and was only taken into account as to whether it was reported or not in the literature in the quality assessment of this review. There were a few studies using triathletes and marathons and/or triathlons as the exercise protocol but most of the investigations did not report the training status of participants. This is important as trained

athletes maybe less susceptible to oxidative stress due to their enhanced expression of antioxidant enzymes and up-regulation of repair systems, acquired from previous training [77]. Across studies, the time of post-exercise measurement ranged from immediately post-exercise to 28 days following exercise (**Table 2**). However, in most studies, DNA damage was measured immediately post-exercise. Although this was further investigated by analysis of subsequent time-points, a significant increase at some of those time-points may not have been found, due to a smaller sample size.

4.6 Future Research

A relatively new biomarker has been used recently, the γ -H2AX, to assess DNA double-strand breaks in cancer research [94]. This assay is considered a sensitive method of measuring DNA damage, due to its ability to detect very low levels of double strand breaks, which the comet assay could not otherwise detect [107]. Lippi *et al.* reported an increase in DNA injury, associated with running distance and intensity, with γ -H2AX foci analysis in lymphocytes. Amateur runners completed a 5-km, 10-km, 21-km and 42-km running trial on 4 separate occasions. The authors observed a small increase in γ -H2AX foci after both 5-km and 10-km of running, a larger increase after 21-km and an even larger increase after 42-km, indicating a dose-dependent relationship of DNA damage with distance and intensity [108]. This method could represent a salient methodological approach for future research to better address the complexity of exercise and DNA damage. Similarly, although challenging, incorporating direct free radical detection in parallel studies may yield more robust results and sensitive data. Lastly, the role of antioxidant supplementation and its potential effects on DNA damage following exercise could be the next focal point of future meta-analyses. As a final practical aspect of performing subsequent meta-analyses, future authors are recommended to include all numeric values in text for easier extraction.

4.7 Conclusions

This systematic review and meta-analysis demonstrated a large increase in DNA damage immediately following an acute exercise bout as well as after 2 hours and up to 1 day post-exercise, while such an increase was not evident measured between 5-28 days. Furthermore, only studies using the comet assay showed significance, compared to 8-OHdG. The analysis further showed that high-intensity exercise results in an increase in DNA damage, suggesting that greater DNA damage may be positively associated with increasing exercise intensity in a dose-dependent manner, while no significance was observed in the long-distance studies, possibly due to the initiation of repair systems during such events. However, due to limitations discussed and the paucity of evidence for most secondary outcomes, findings should be viewed with a degree of caution. Although an increase in DNA damage occurs after exercise, this is not necessarily a negative outcome *per se*. Such damage is most likely repaired within 3 days, or likely even sooner, as the long-distance studies may suggest, and thus may be transitory and should not confer any long-term adverse health outcomes on the individual or athlete. However, this will differ across individuals due to variation in individual thresholds since there are multiple factors to consider as explained in (but not limited to) in the multi-dimensional model. The hormesis curve describes, in a somewhat one-dimensional manner, how exercise modulates any advantageous or harmful effects through RONS by increasing DNA oxidation between the two-end points of the curve, physical inactivity and overtraining. Finally, the proposed multi-dimensional model may allow for a better understanding of the complex and multi-factorial relationship between DNA damage and exercise.

Declarations

Data Availability

The data presented in this systematic review is available in the associated studies, and references are provided.

Compliance with Ethical Standards

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Conflict of Interests

The authors Despoina V. Tryfidou, Conor McClean, Michalis G. Nikolaidis and Gareth W. Davison declare they have no conflicts of interest relevant to the content of this review.

Authors' contributions

Despoina V. Tryfidou performed the data search, selection, and analyses. Despoina V. Tryfidou, Conor McClean, Michalis G. Nikolaidis and Gareth W. Davison all contributed to writing, discussion, and final approval of all results and the manuscript.

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